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Environmental Stress Pathway Project

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VIMSS Virtual Institute for Microbial Stress and Survival | Massachusetts Institute of Technology | Missouri | University of Washington | Montana State University | Southern Illinois University Carbondale | Sandia National Laboratories | Oak Ridge National Laboratory | Berkeley | http://vimss.lbl.gov/

Application of Proteomics and Lipid studies in Environmental Biotechnology

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Joint Bioenergy Institute
Lawrence Berkeley National Laboratory

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ACKNOWLEDGEMENT

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Microbial metal reduction

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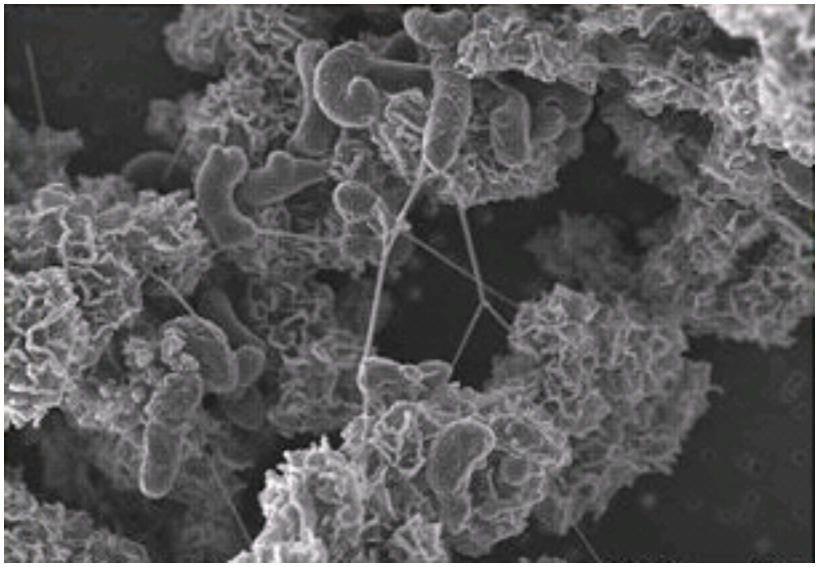
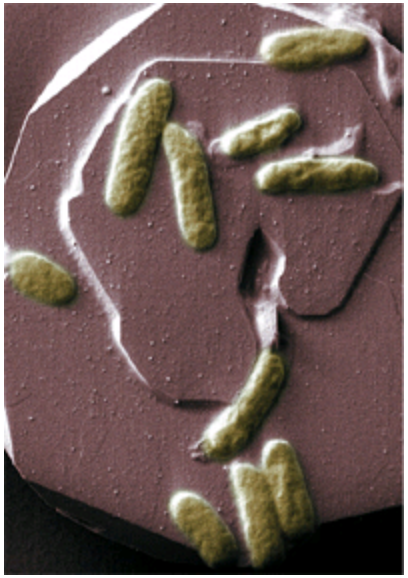


Desulfovibrio vulgaris



Shewanella oneidensis

$U^{(VI+)}$ —Microbial metal reduction→ $U^{(IV+)}$

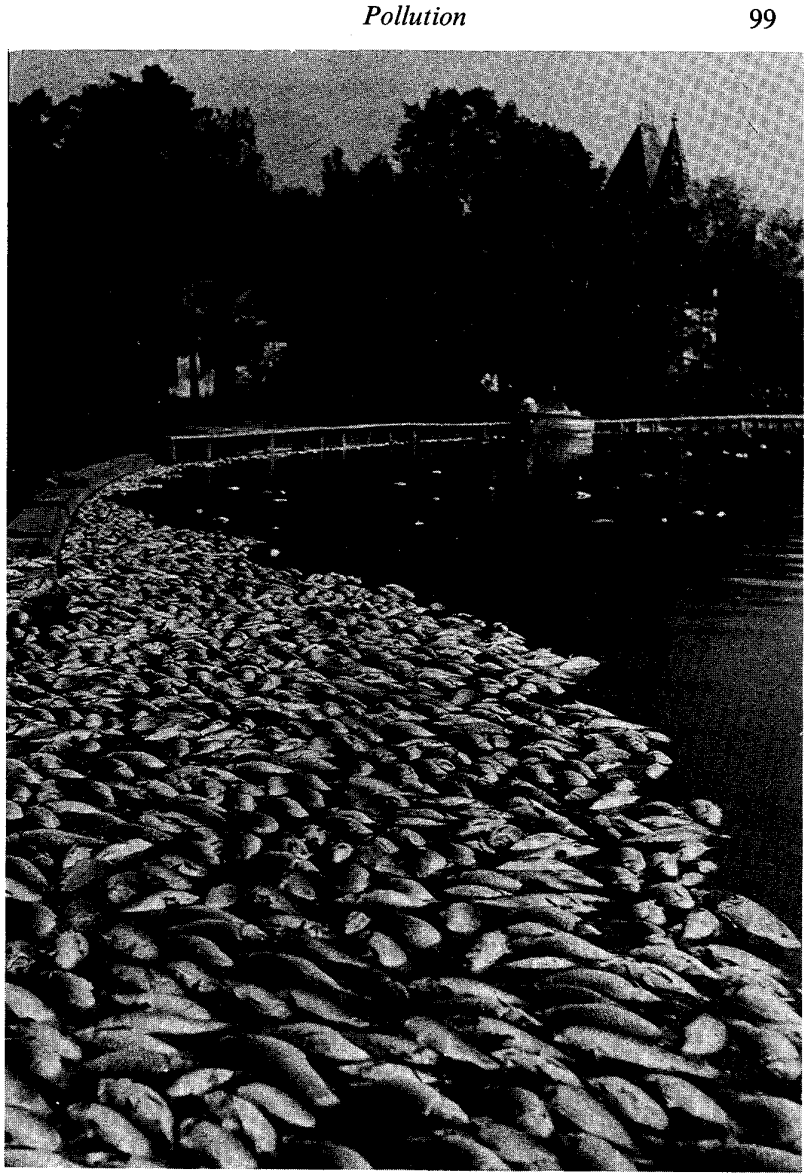
(Soluble in water)

(insoluble mineral uraninite)

Figure 1: *Desulfovibrio vulgaris* Biofilm on metal surface (Matthew Fields, Montana state university)

Figure 2: *Shewanella oneidensis* CN32 cells on the surface hematite (Alice Dohnalkova, Pacific Northwest National Laboratory)

Sulfate and Metal reduction: Biofouling



Pollution 99

Fig. 16. Fish killed by sulphide pollution. Dead carp killed by biogenic H_2S as a result of pollution in Lake Palic, Yugoslavia, in 1971 (courtesy of Dr R. Vamos).

- Biomagnification of mercury – Methyl mercury formation.
- Biocorrosion
- Biofouling of crude oil

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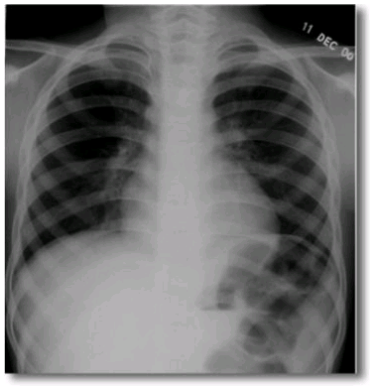
Radioactive and heavy metal waste management

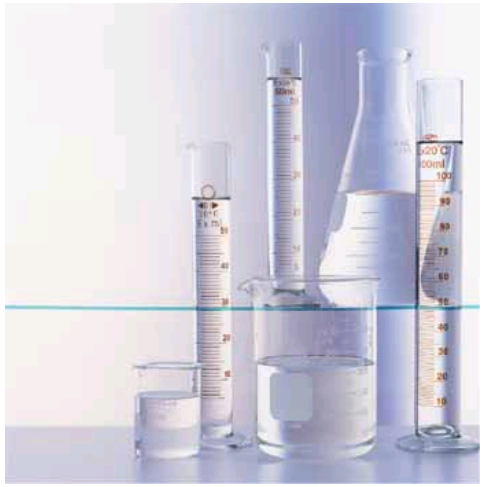
Mining

Stored legac

Power Plants



Medicine



Research

Figure credits
www.greatbasinminewatch.org
www.fas.org/irp/imint/doe_hanford_fftf_01c.jpg
http://nc.sierraclub.org/images/NuclearPowerPlant.jpg

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Desulfovibrio vulgaris Hildenborough

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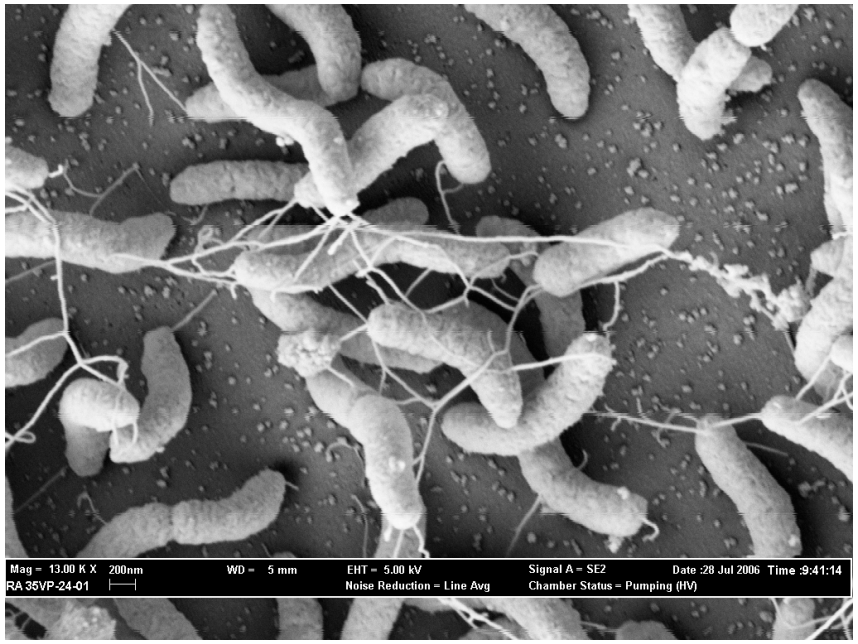

- Sulfate reducing bacterium
- Anaerobic organism
- Found in heavy metal and nuclear waste sites
- Genome was sequenced in 2003

Heidelberg Nat. Biotechnol **22:**554-9

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Using Environmental Microbes

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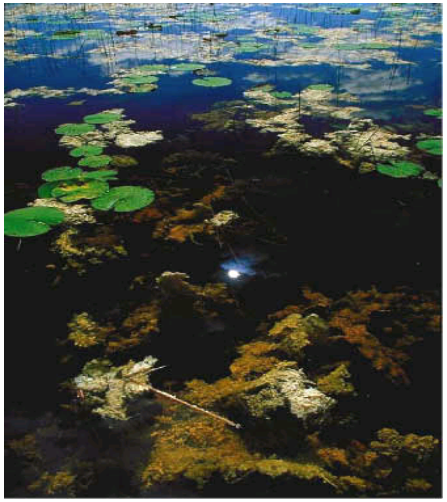
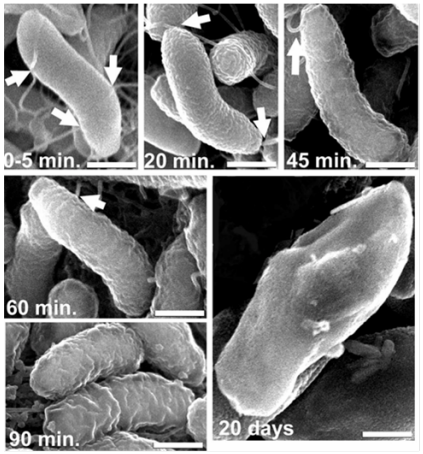


Understand the affect of environmental factors on bioremediation potential of *D. vulgaris*

Apply this knowledge to accelerate the bio-containment of heavy metal waste or limit adverse impact of such organisms in the environment



Exposure to Air

Physiologically Relevant environmental stresses

- Salt
- pH
- Oxygen
- Nitrate
- Heavy metals

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Cell wide data ??

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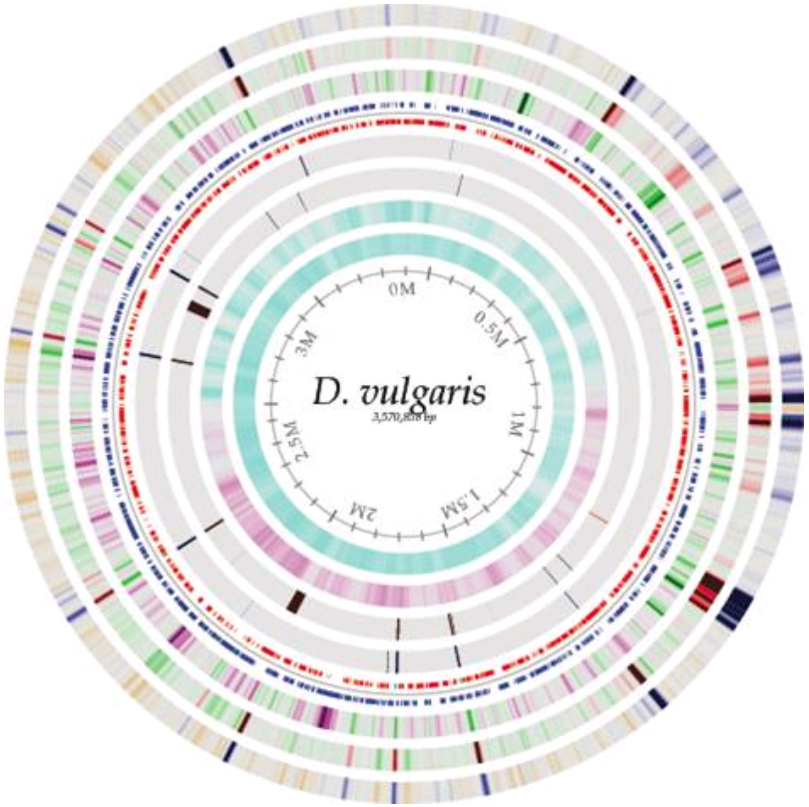

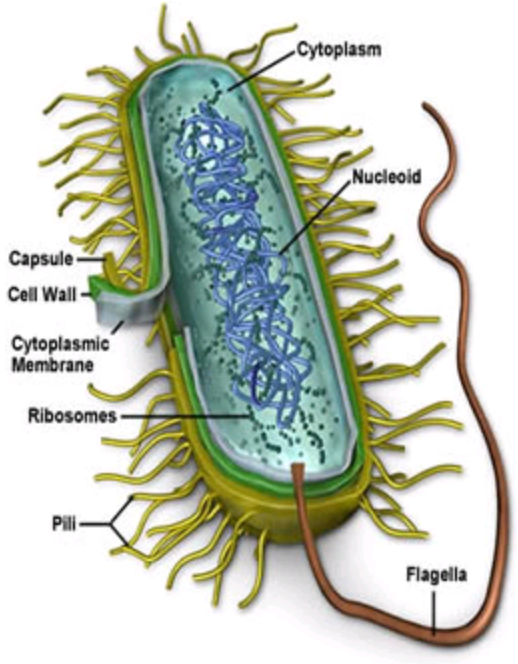

Genome sequence indicates the presence of 3480 genes

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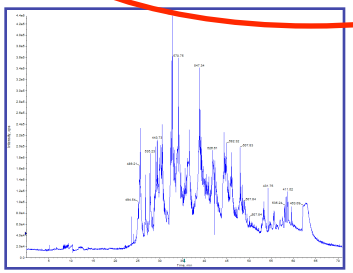
Functional genomics pipeline

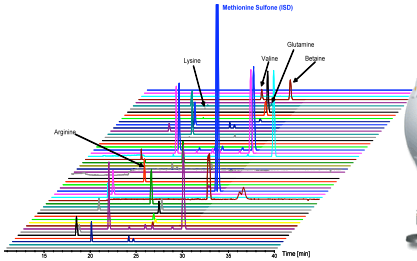
Biomass production

Transcripts

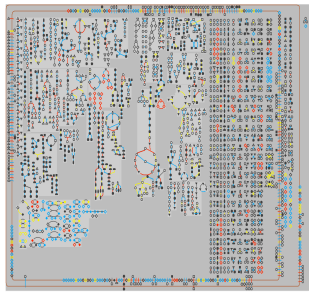
Proteins/ lipids



Metabolites



Analysis



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Why proteomics?

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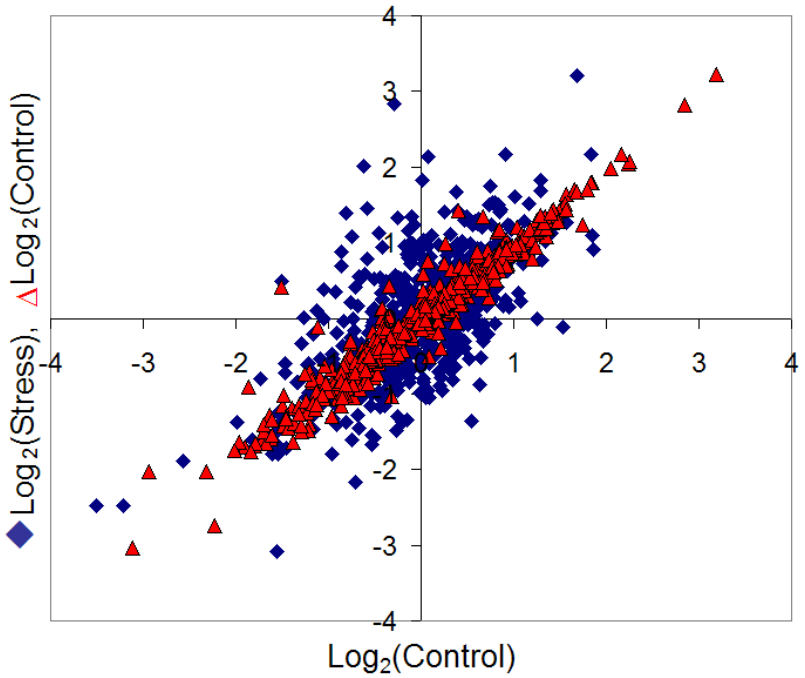
Proof of expression/ Change

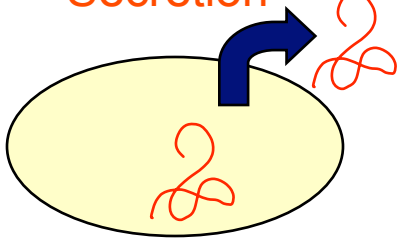
Differential Expression:



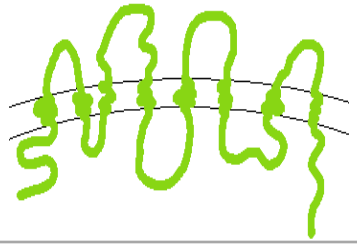
Activation State

Localization:

Secretion

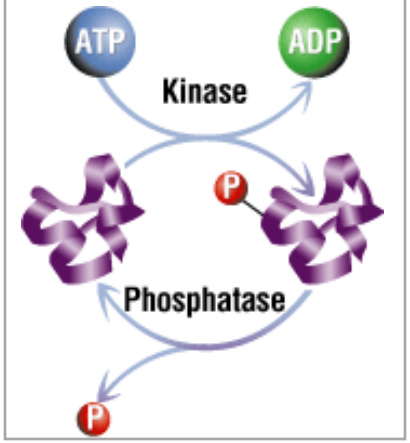


Membrane Interaction

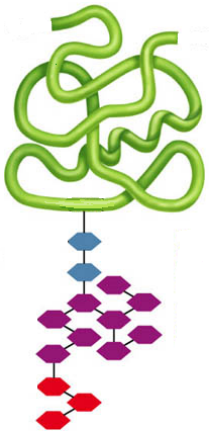


Post translational modifications:

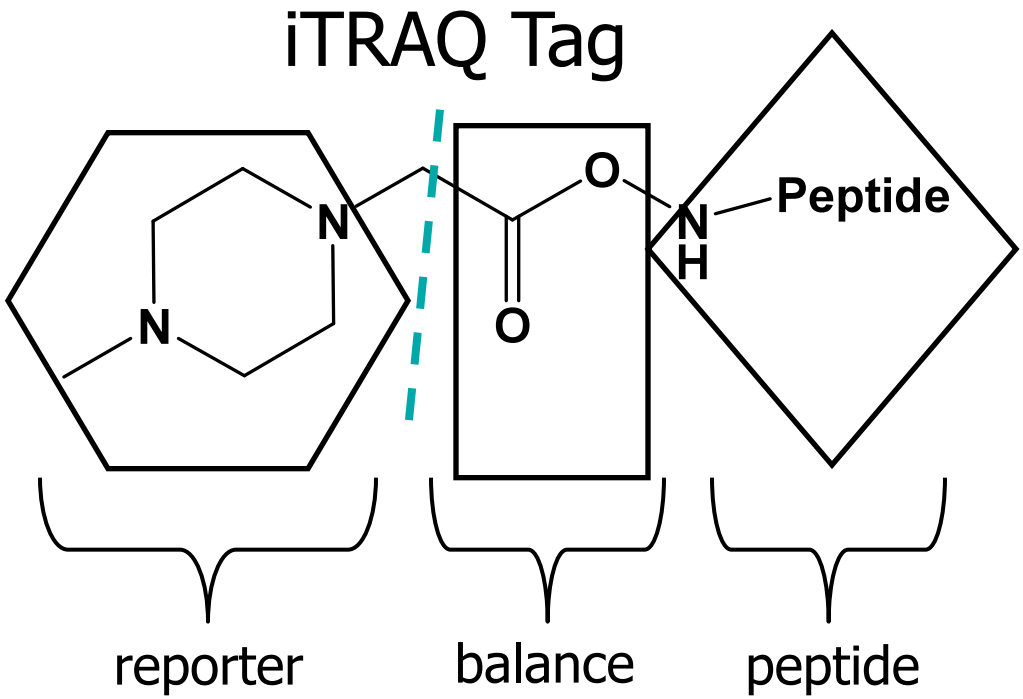
Phosphorylation



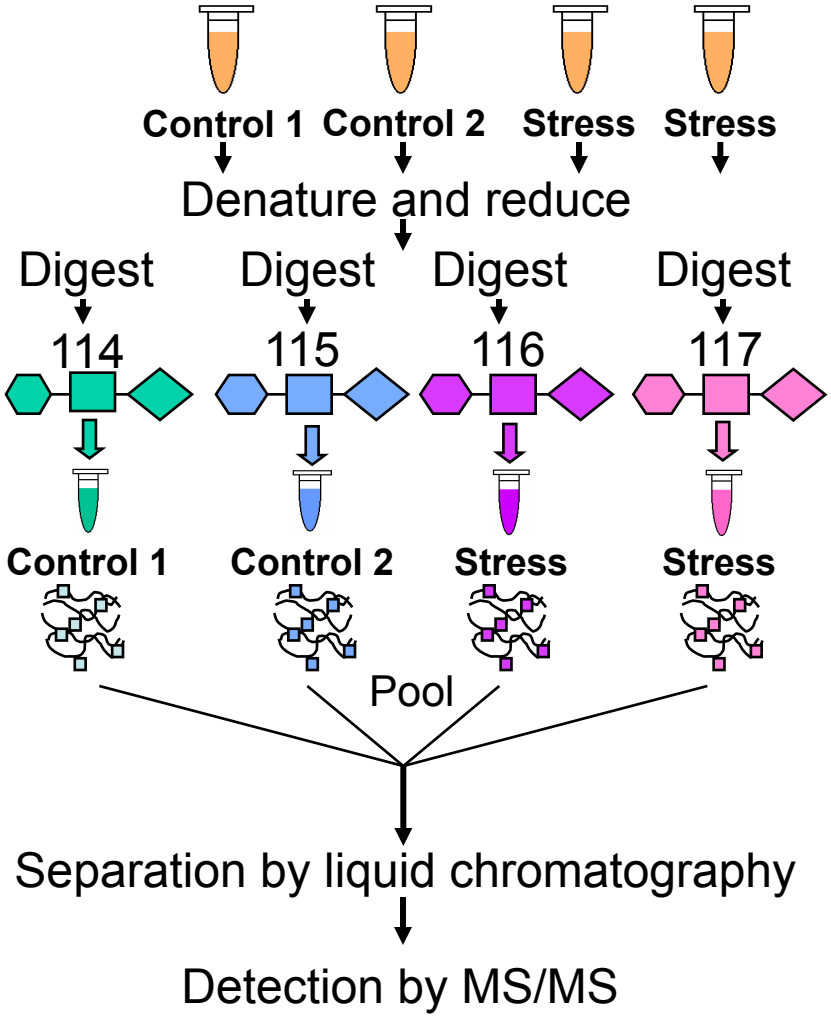
Glycosylation



iTRAQ Peptide Labeling Strategy



Mass	Reporter	Balance
114	^{13}C	^{13}C, ^{18}O
115	$^{13}C_2$	^{18}O
116	$^{13}C_2$, ^{15}N	^{13}C
117	$^{13}C_3$, ^{15}N	--



Ross PL, et al., Mol Cell Proteomics, 2004; 3(12):1154-69

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Tandem LC-MS Proteomics

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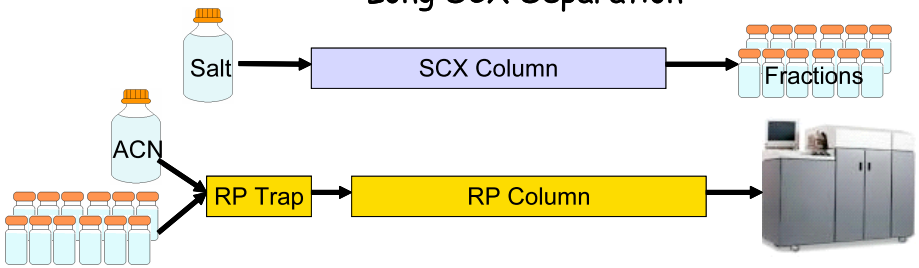
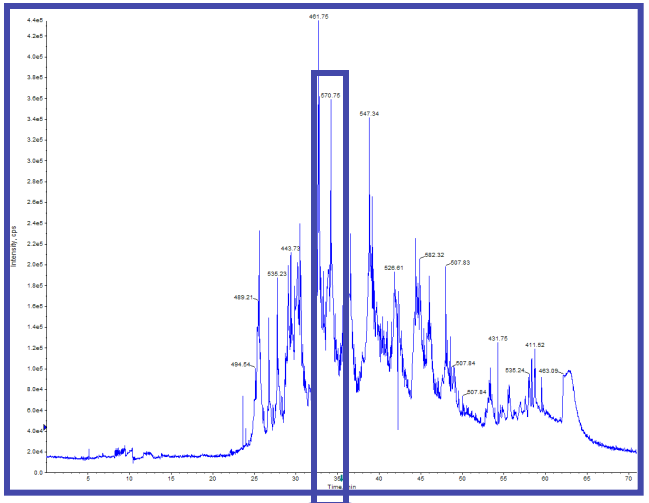
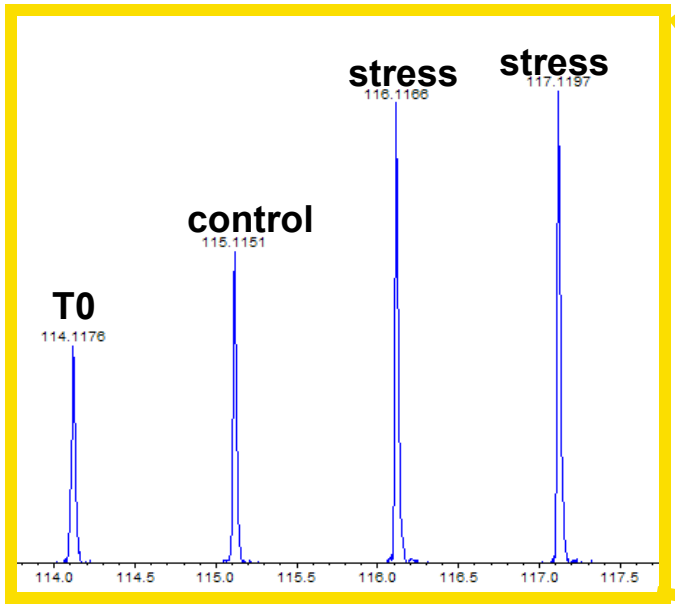
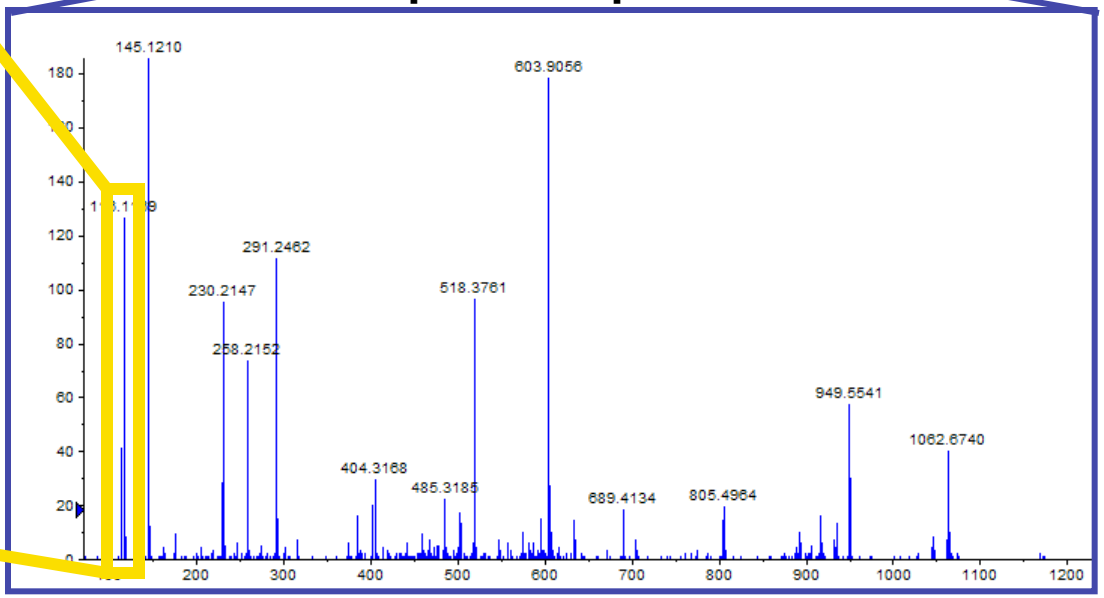




Quantitation



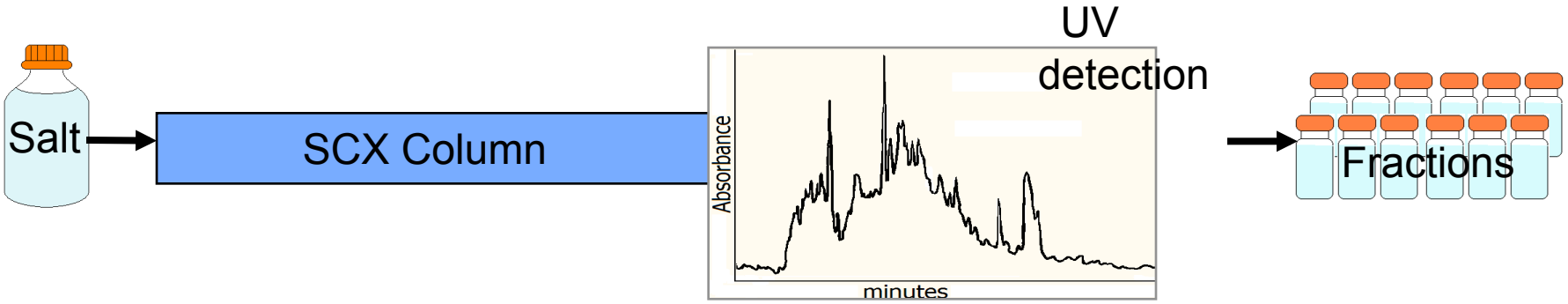
Peptide Sequence



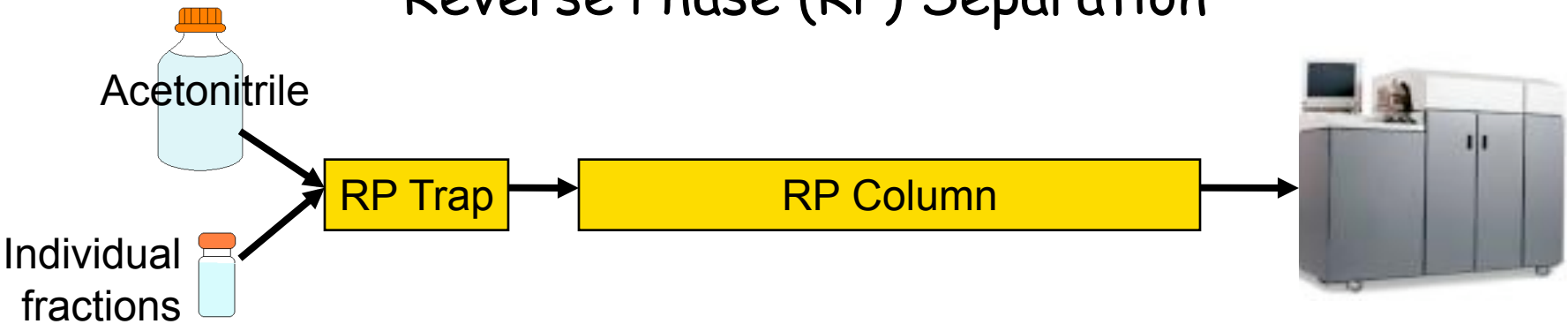
mass/charge

2-D Liquid Chromatography

Strong Cation Exchange (SCX) Separation



Reverse Phase (RP) Separation



Wolters DA, Washburn MP, Yates JR, 3rd. Anal Chem, 2001; 73(24)):5683-90

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Comparative Proteomics

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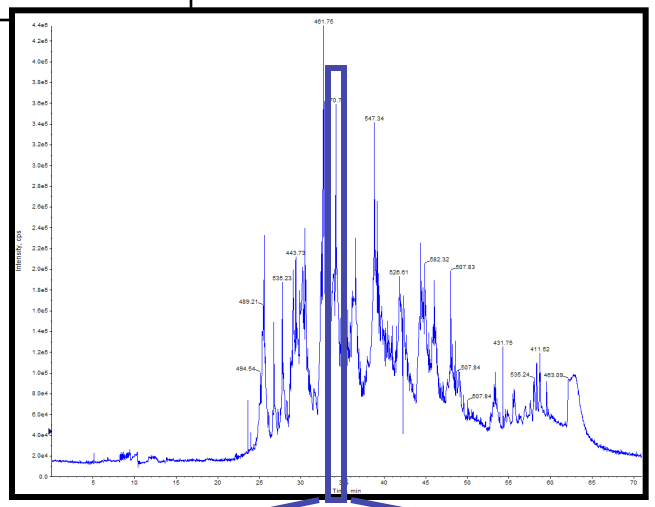
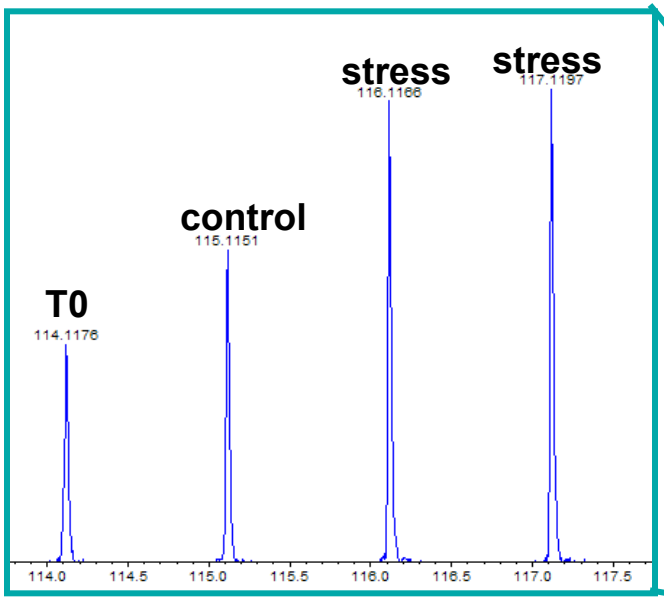
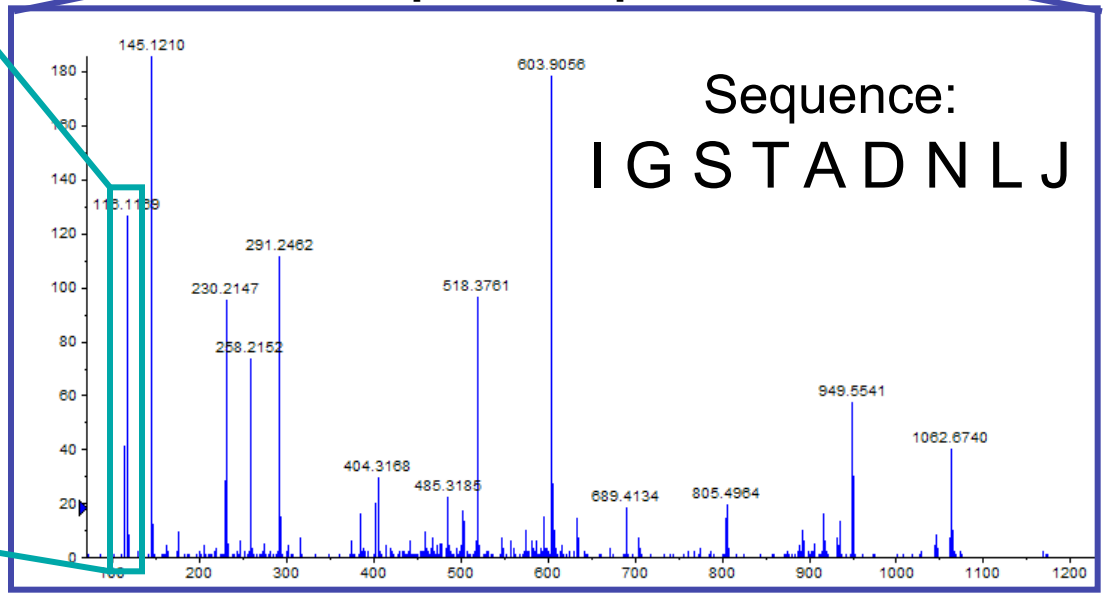
Total Ion Chromatogram



Quantitation

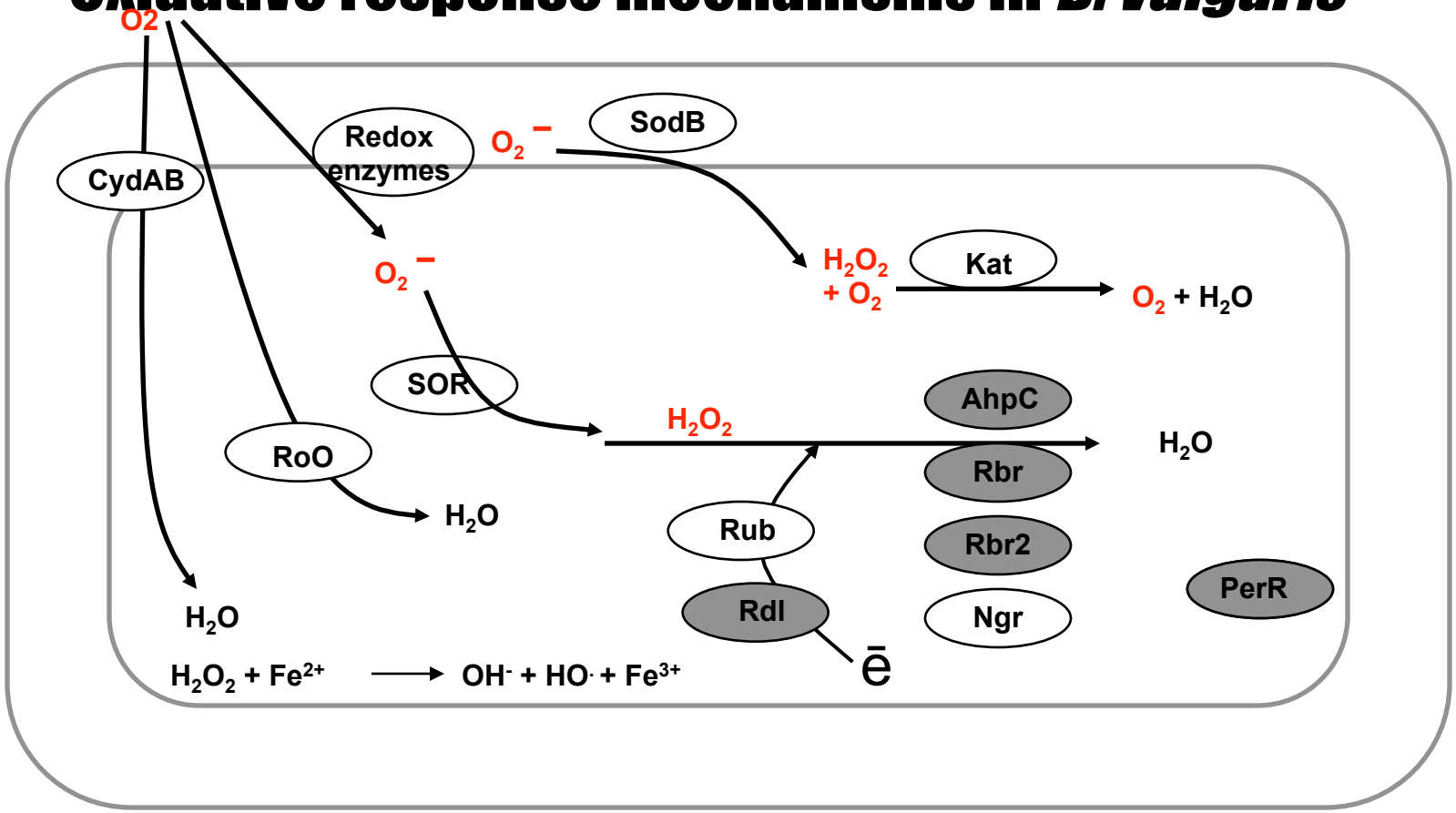


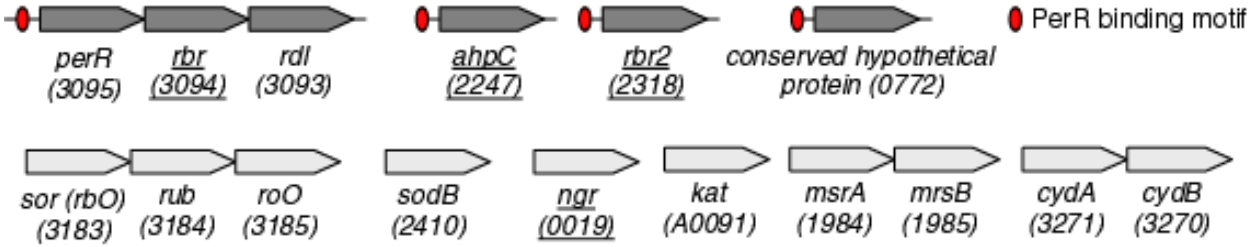
Peptide Sequence



mass/charge

Oxidative response mechanisms in *D. vulgaris*





Lumppio et al J Bacteriol (2001) 101-8; 183
Dolla et al J Biotechnol **126:**87-100
Rodionov Genome Biol **5:**R90.

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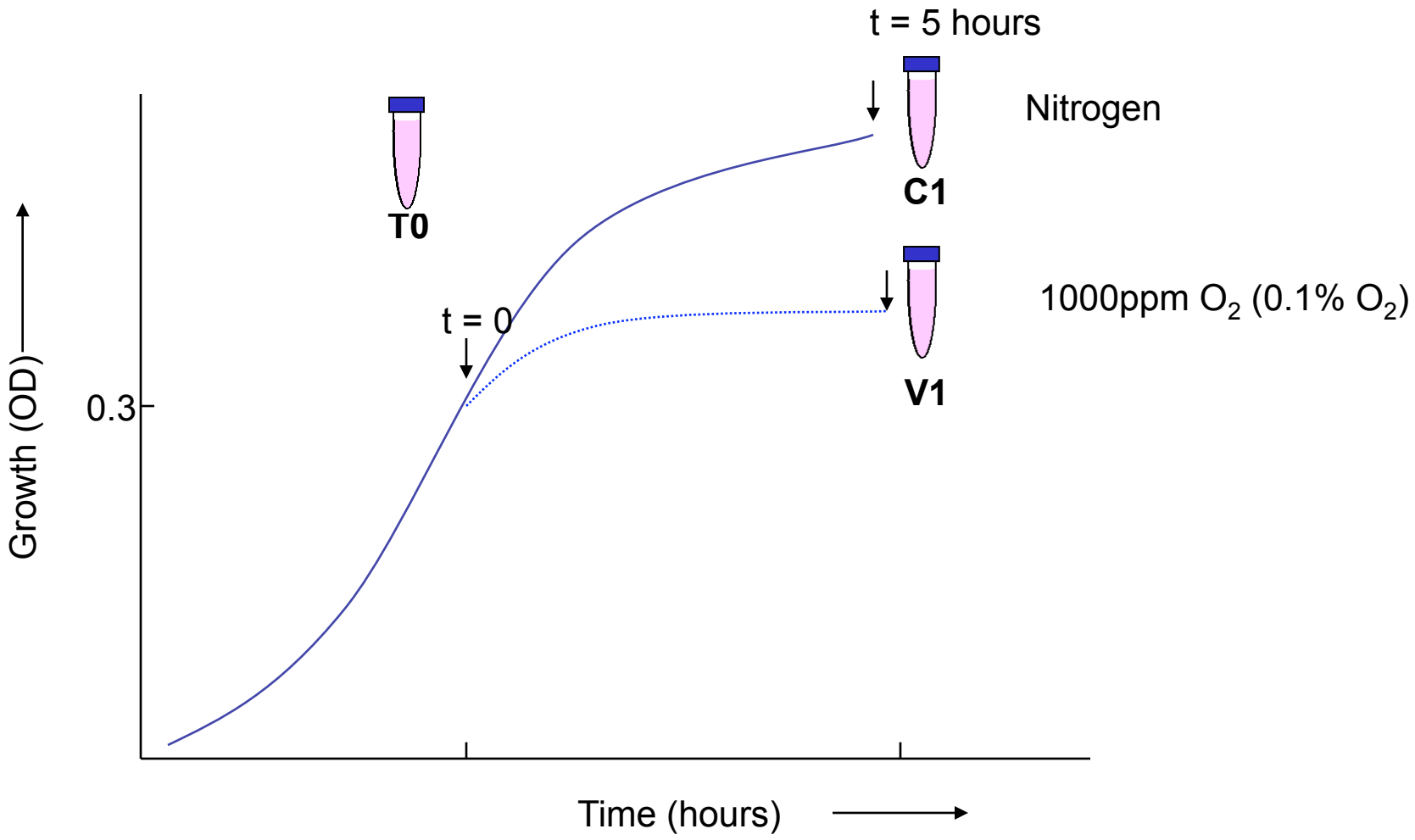
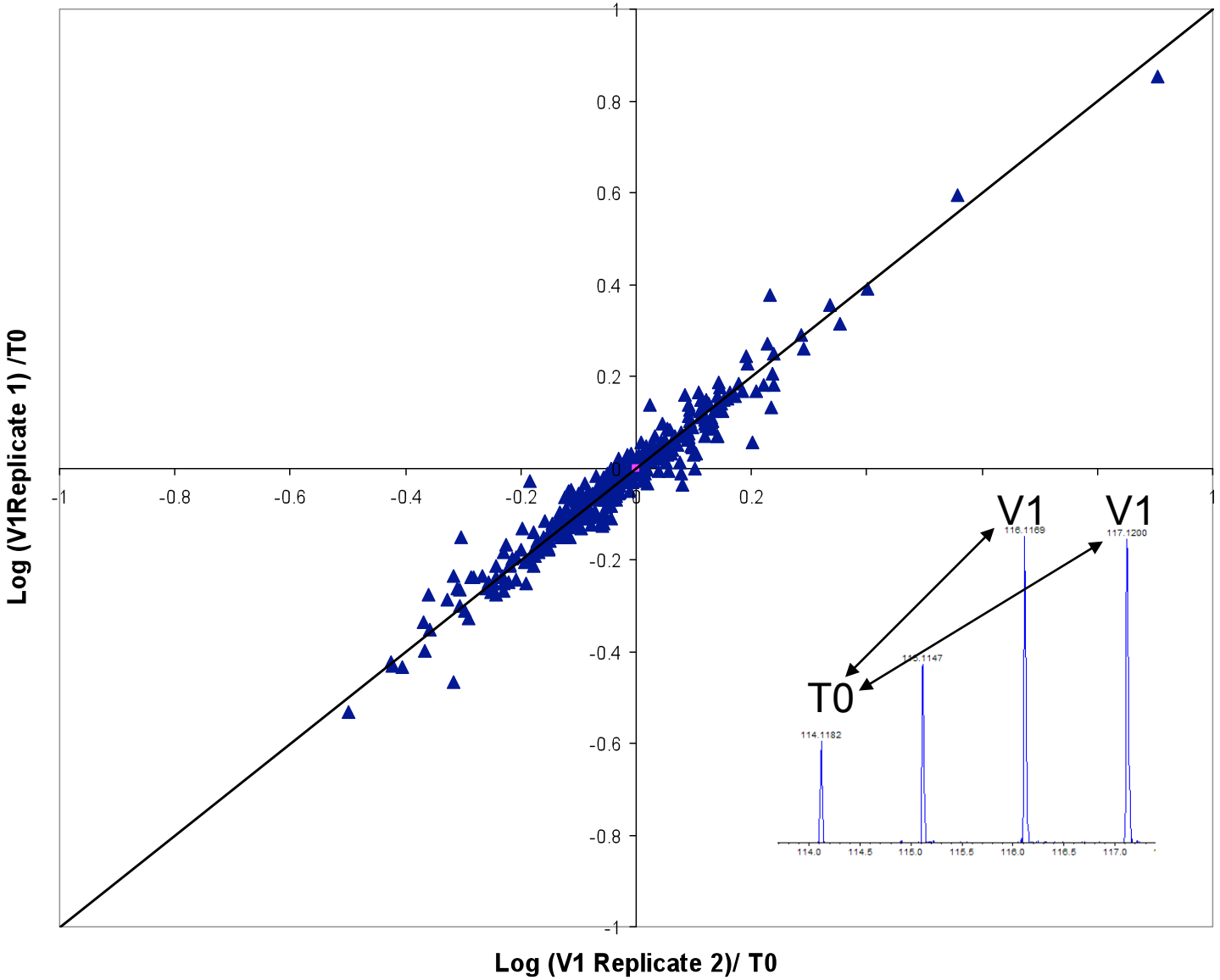
iTRAQ analysis of 0.1% O_2 exposure in *D. vulgaris*



Use of internal replicates



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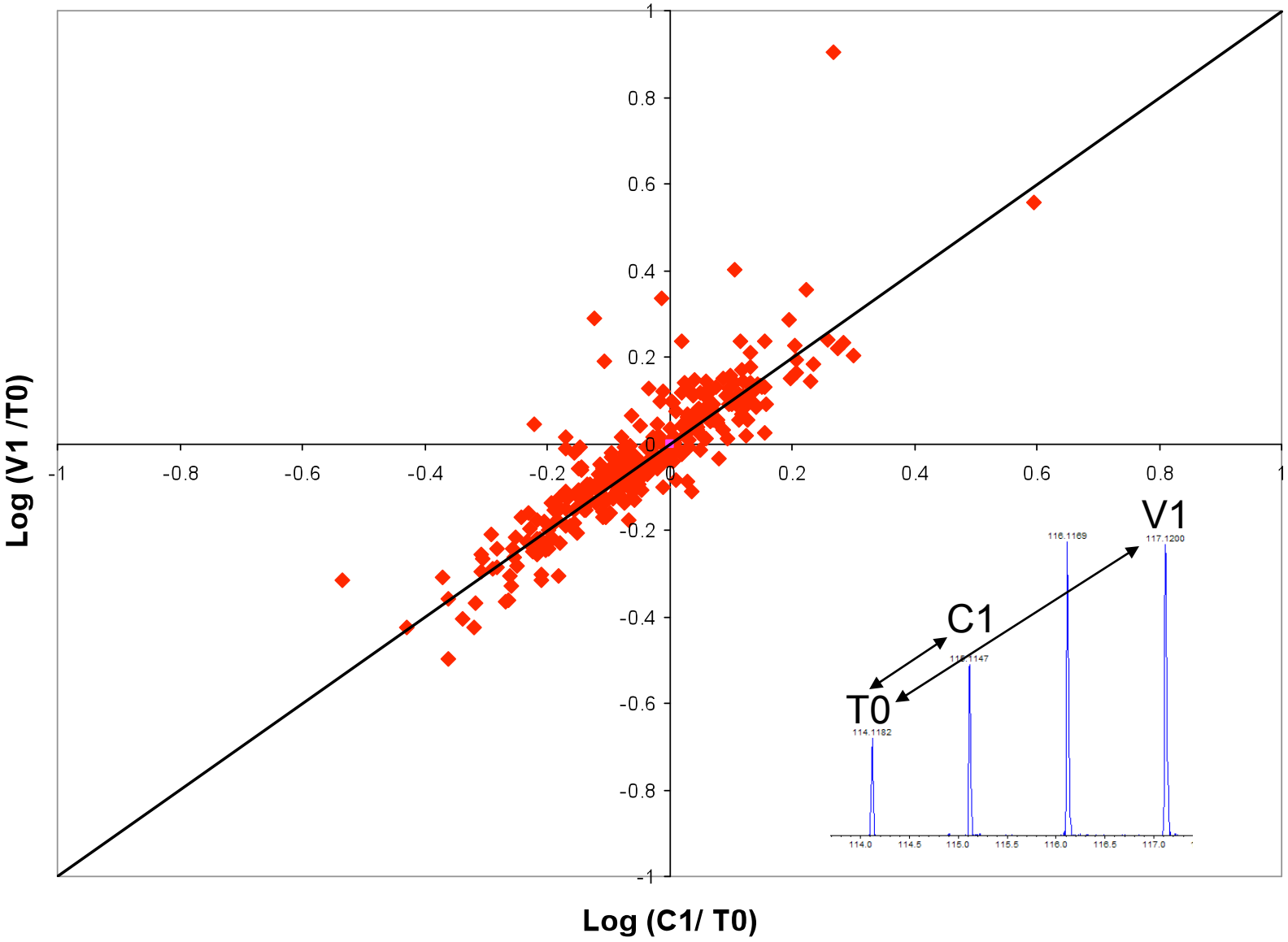
Comparison of Stressed and Control



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Use of error to determine significant changers

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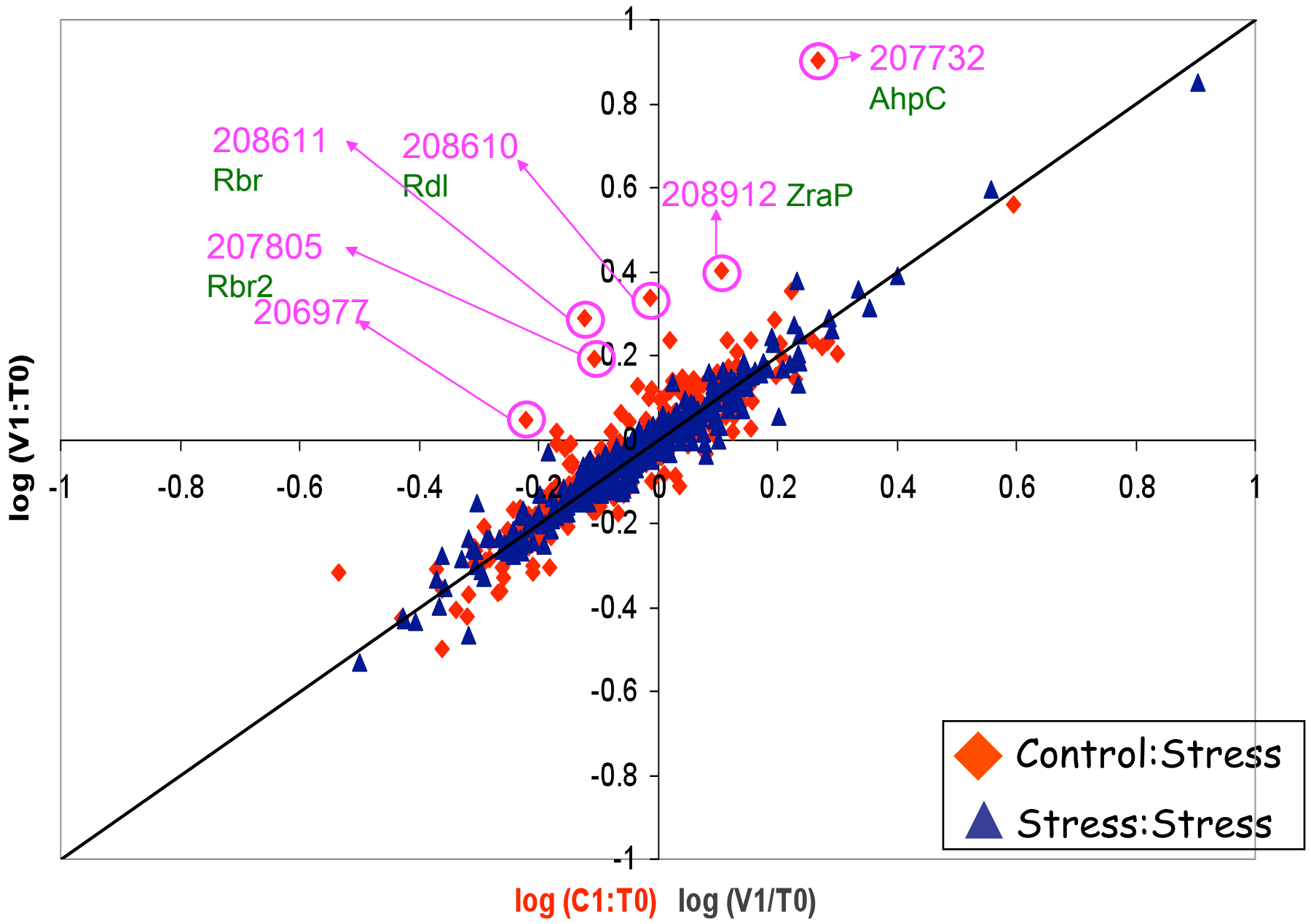
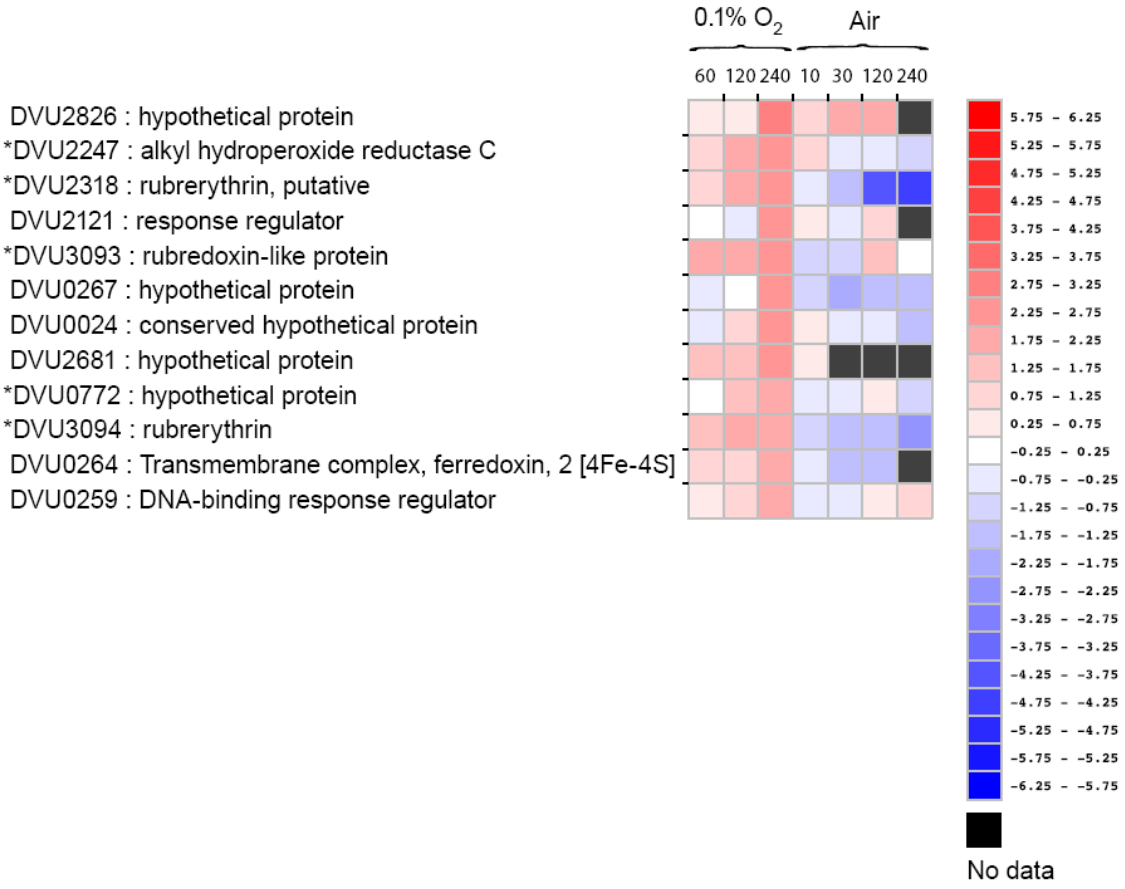


Both transcript and protein data pointed to PerR



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Response to low O_2

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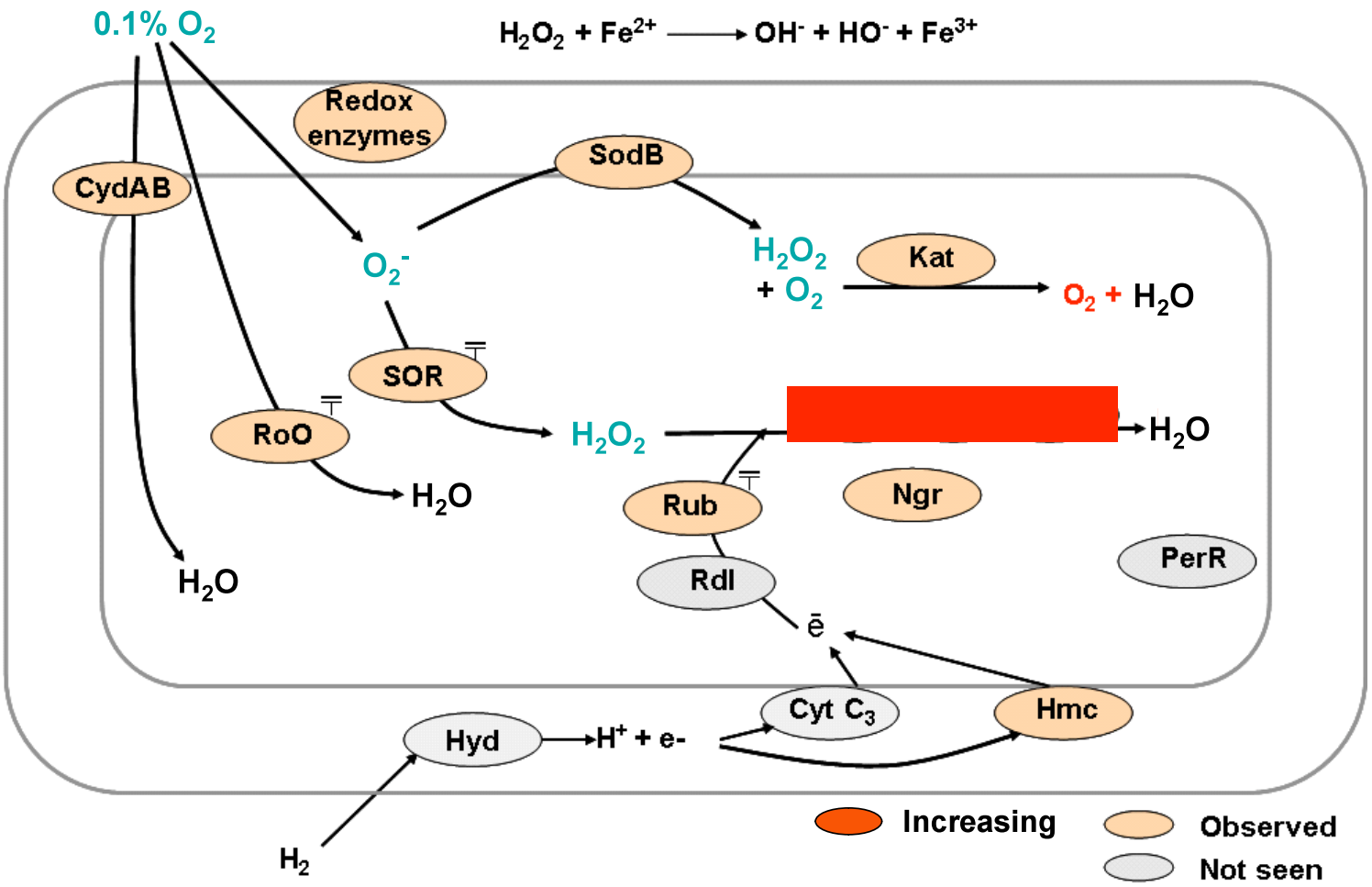

Mukhopadhyay et al J Bacteriol (2007) 5996-6010; 189

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Some interesting hypothesis to follow up on

- *D. vulgaris* adapted to occupy sub-oxic environment ?
- Per regulon in *D. vulgaris* provides additional mitigation over and above SOR and SOD based activity
- The *D. vulgaris* Per regulon may contain additional members than initially predicted
- And can be experimentally tested

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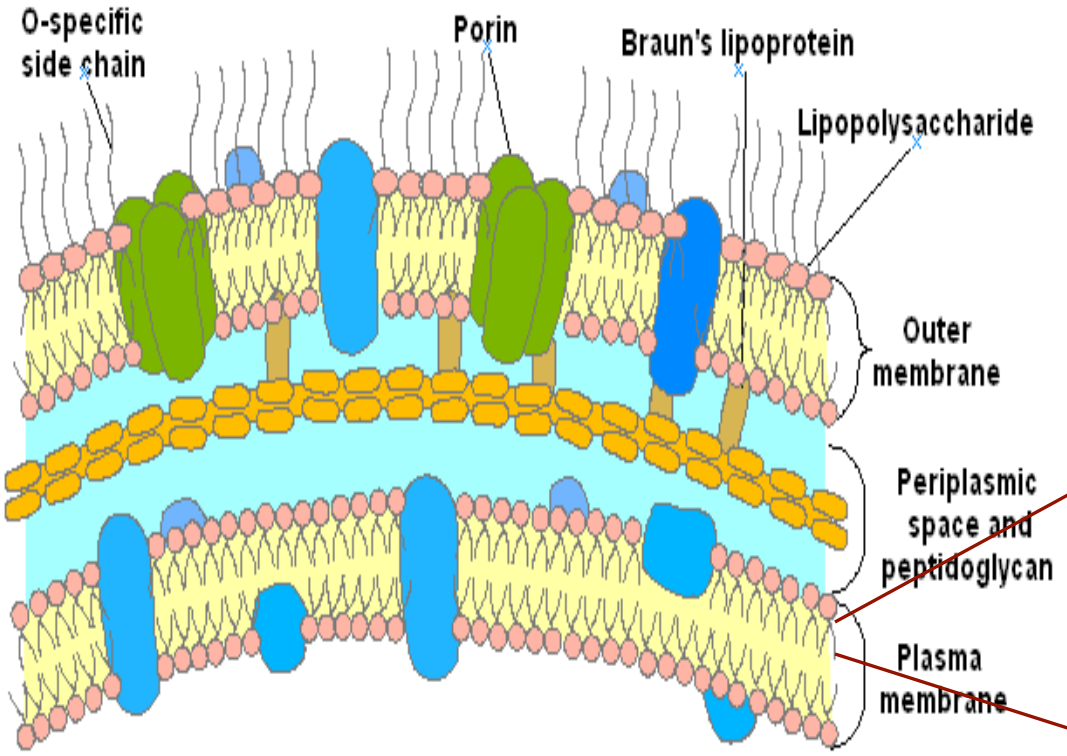
Importance of Cell wall in environmental stresses

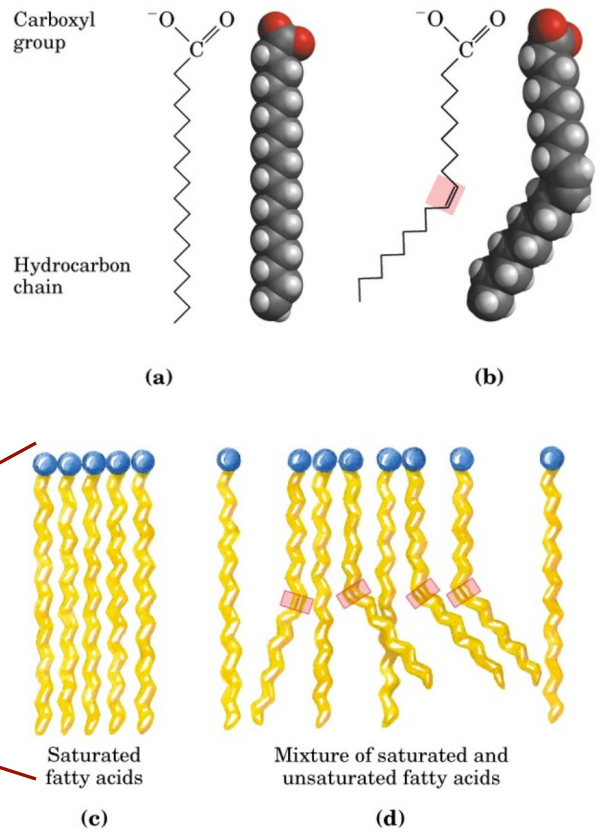
- The cell wall presents the first line of defense for microorganisms
  - Salt stress/ cold stress/ heat/ solvent
  - Optimal homeoviscocity is determined by the distribution of fatty acids in the cell wall and readjusted as the environment changes

Fatty Acid distribution

Schematic of Cell wall (Gram negative)

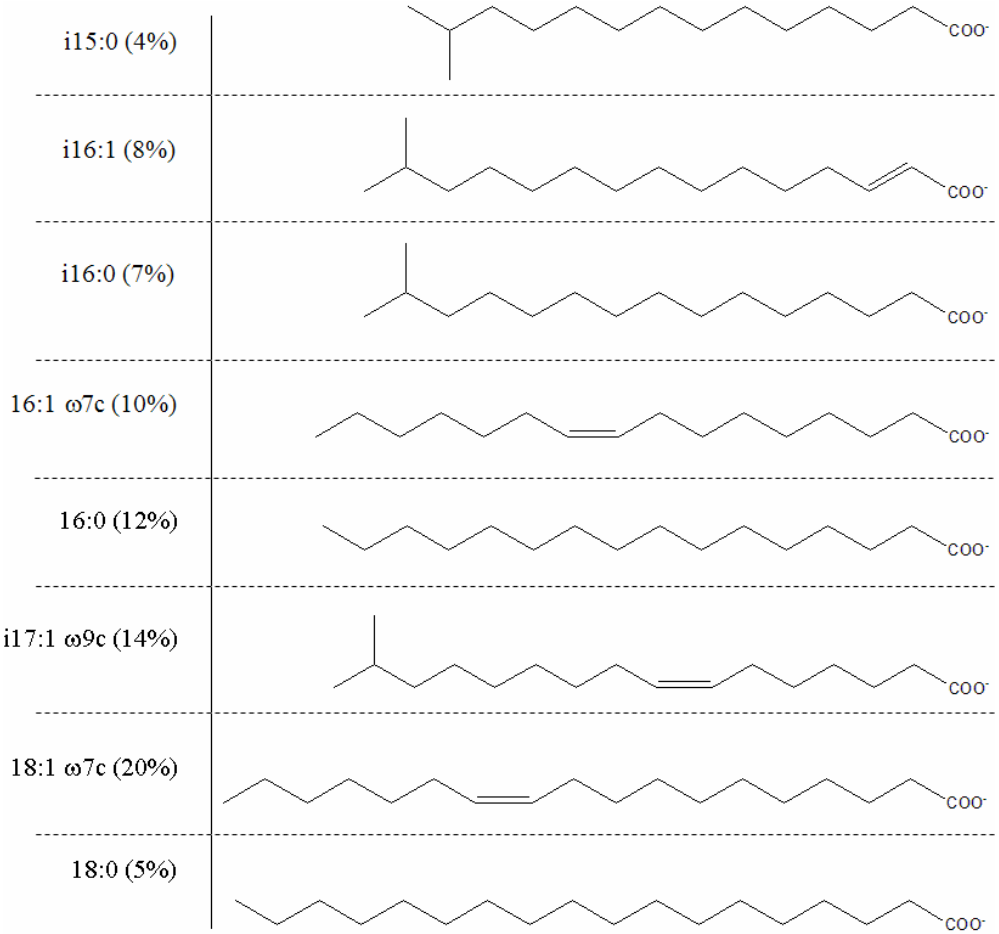


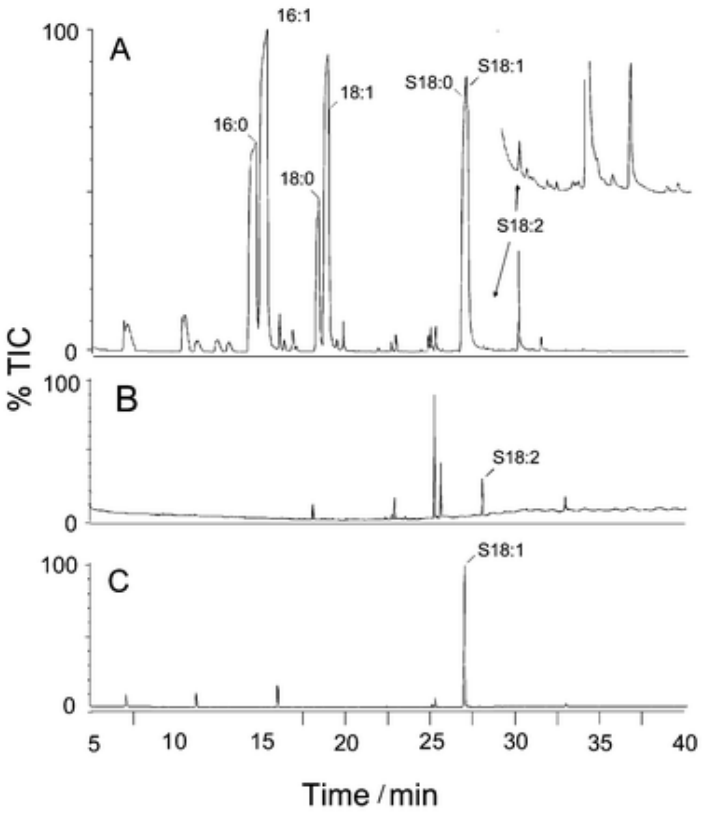
Fatty Acid Structure



FAME analysis for PLFA quantification

PLFA extraction ⟶ Methyl Esterification ⟶ GCMS detection and quantification





Note: The Phospholipid Fatty acid (PLFA) distribution of a microbe is unique

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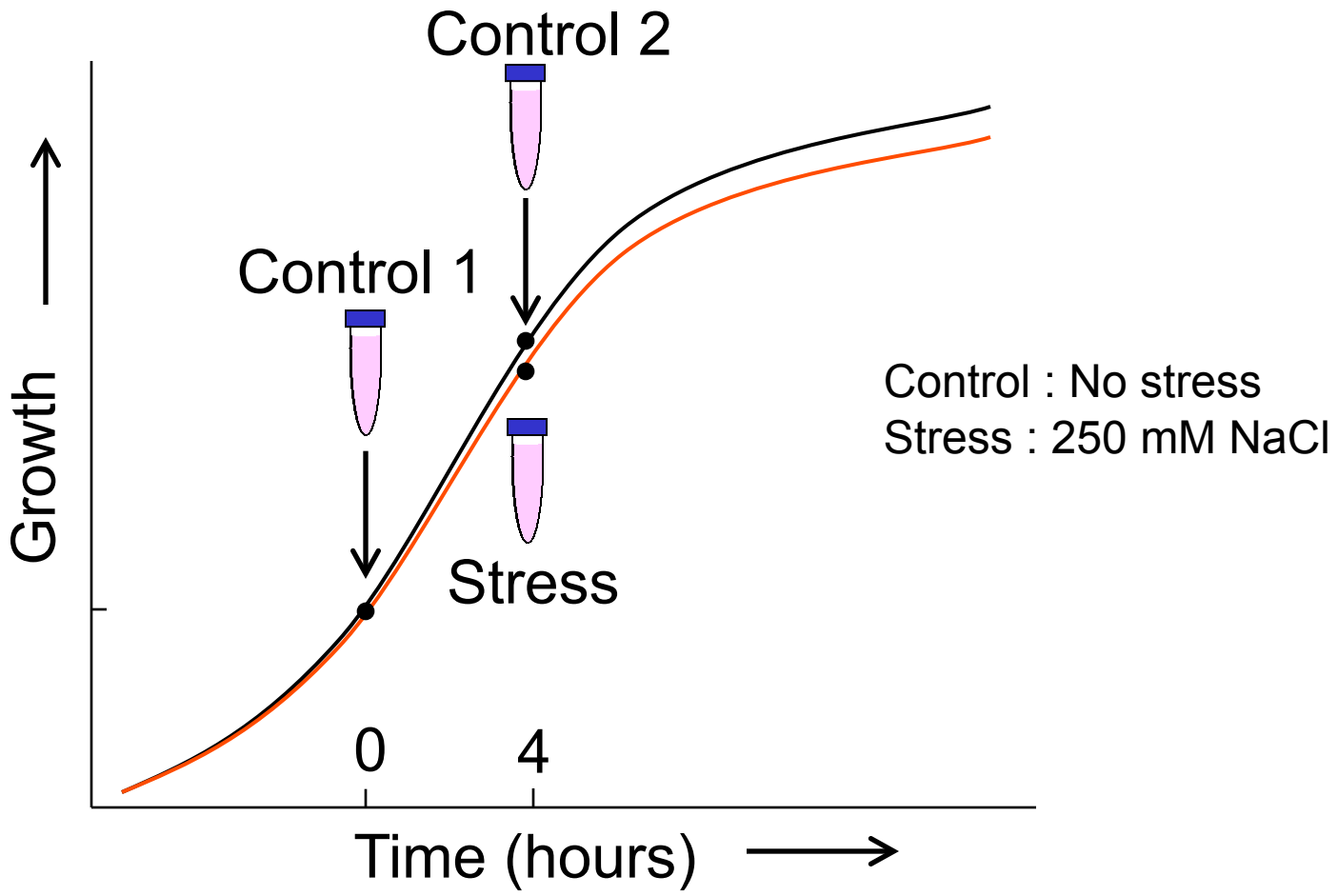
Salt stress in *D. vulgaris*



Control : No stress
Stress : 250 mM NaCl

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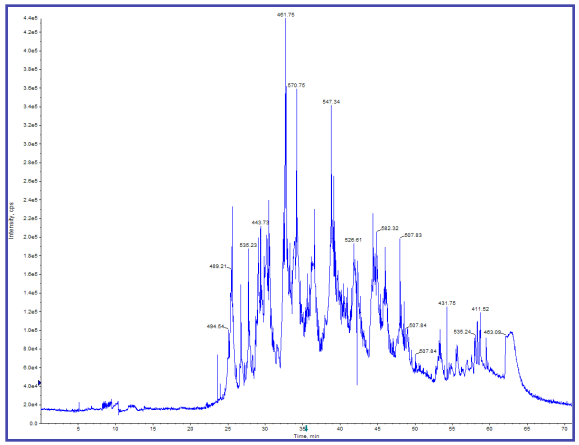
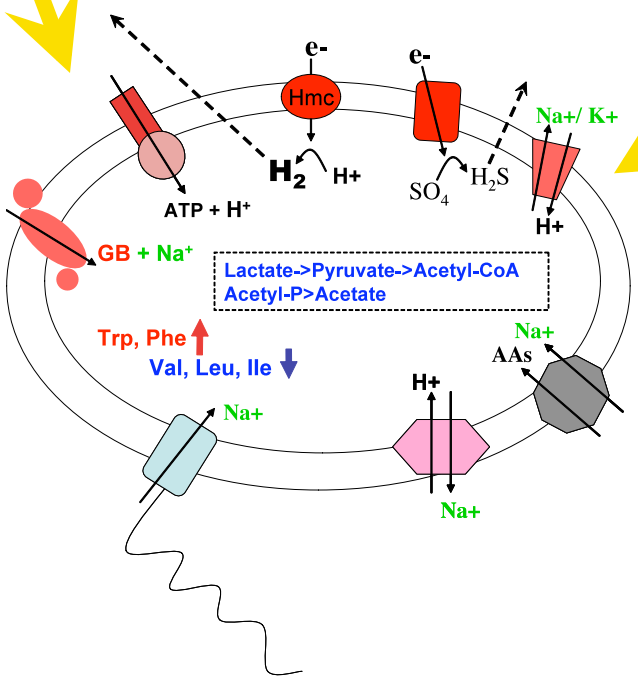
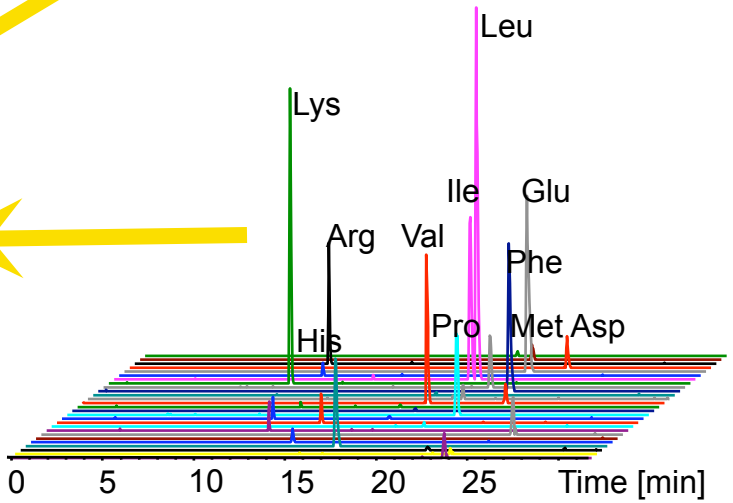
Integrating Functional Genomics

Proteomics



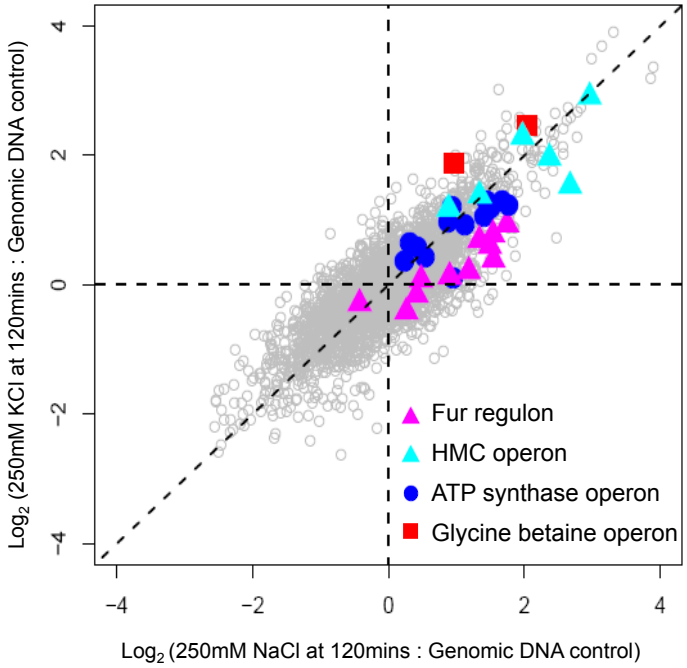
Transcriptomics

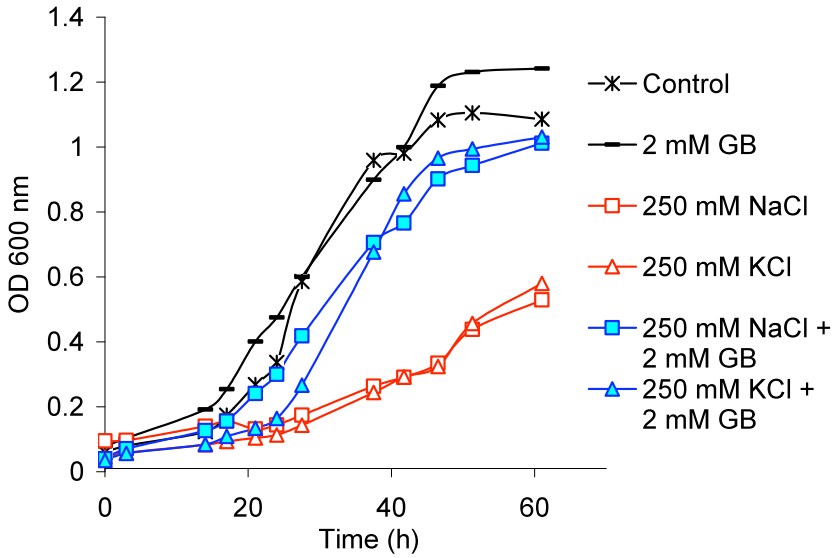




Metabolomics

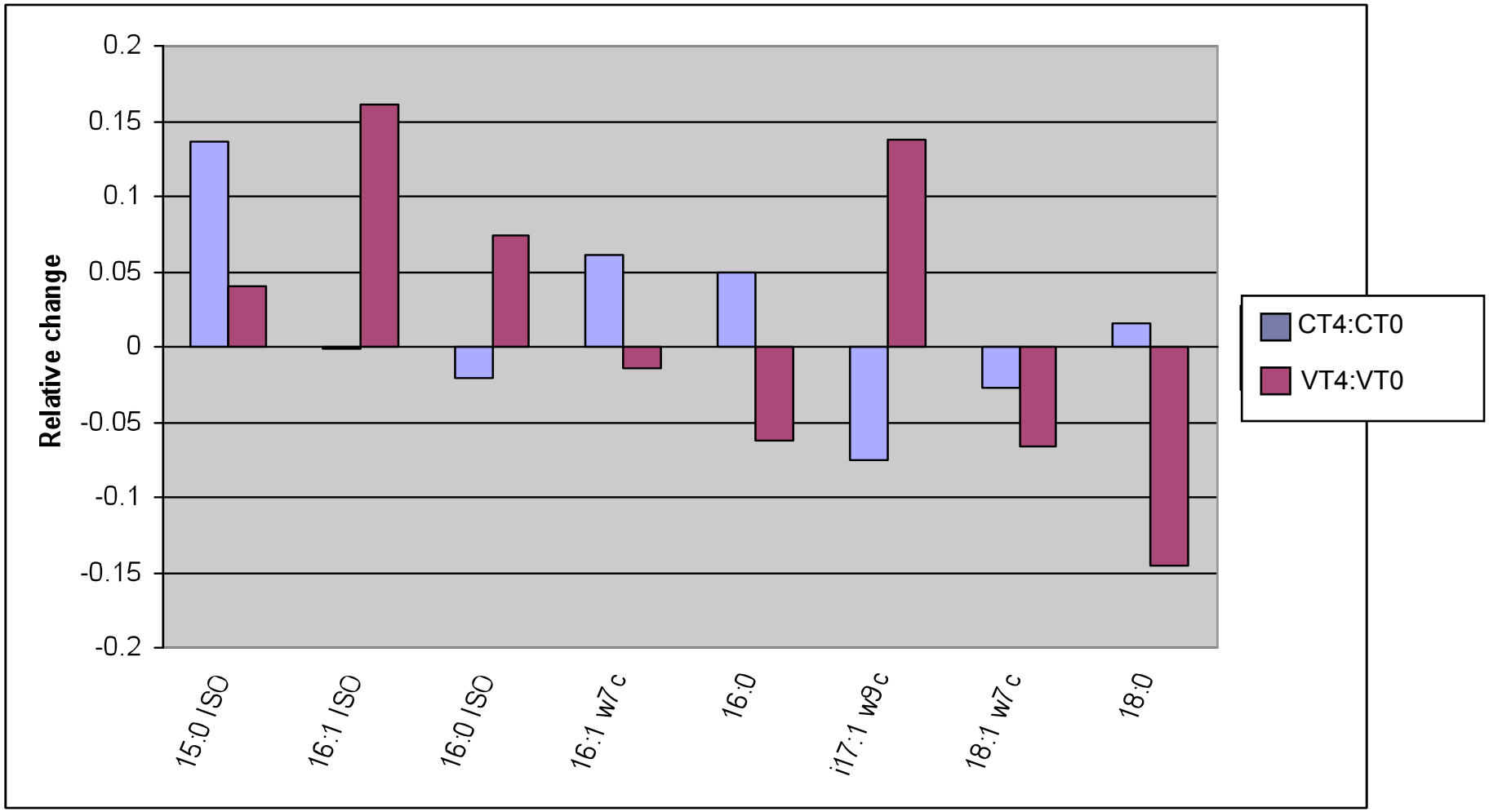
Uptake of Osmolyte is a primary mechanism of stress response





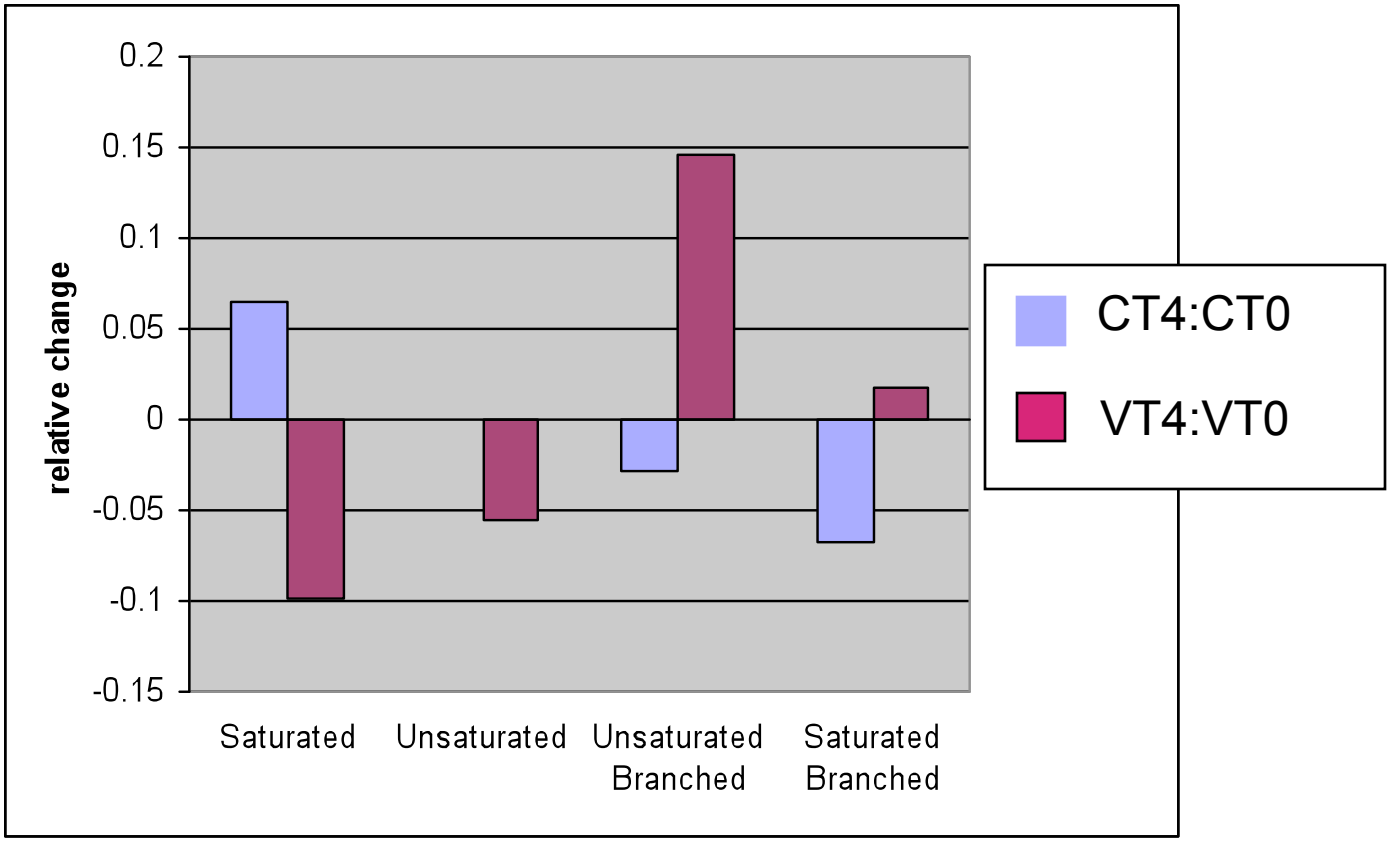
But we were missing some important aspects of cell wide data

Lipid analysis : PLFA studies



Relative change of the 8 major types PLFA after stress

Critical change in PLFA profile



Changes in PLFA to increase fluidity are documented for many bacteria in both Salt and Cold stress.

Increase in branched PLFA also reduces packing and increases fluidity of the membrane

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Salt stress in *D. vulgaris*

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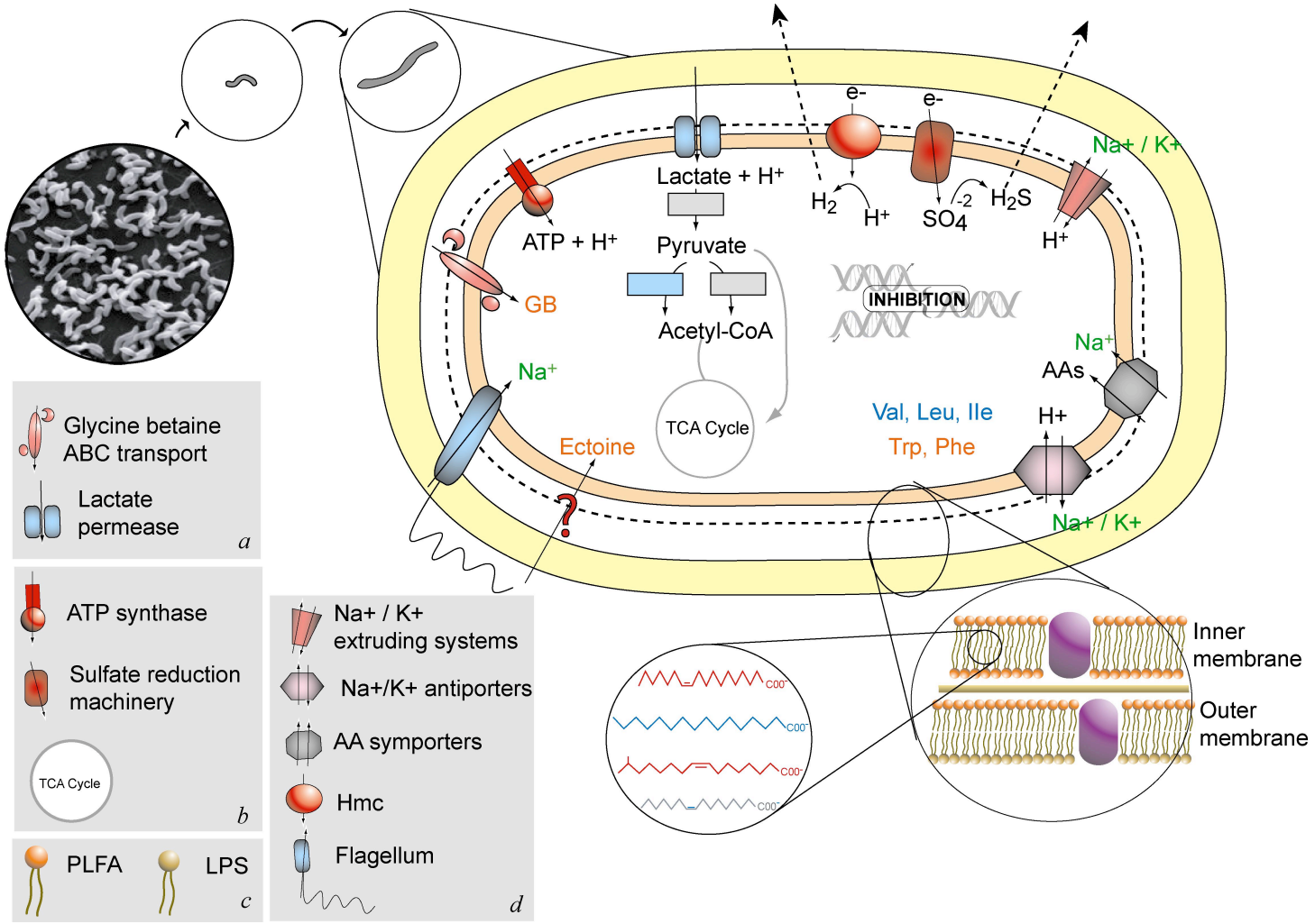

Mukhopadhyay et al J Bacteriology (2006) 4068-4078;188

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PLFA Studies

- Not only is a change in PLFA profile a significant marker of stress response

- PLFA studies can help ID microbes in an ecosystem and provide a excellent HT method to assess the biotechnological potential of a microbial community for a particular function

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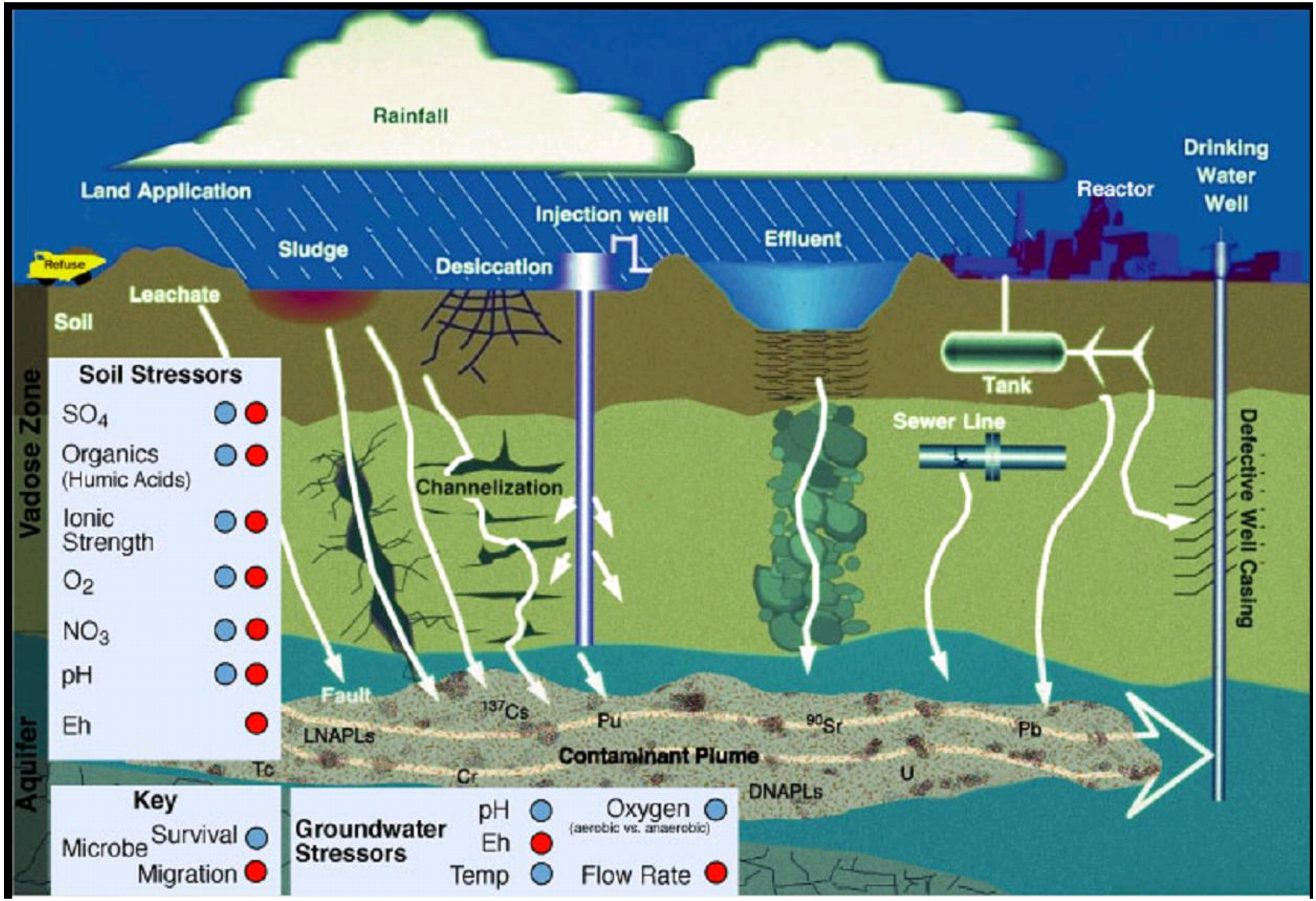
Environmental Stress factors



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Summary

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- Systems biology approaches allow us to obtain cell wide data that can be used to build models for how an organism interacts with its environment.

- These models provide the foundation to build bio-remedial approaches in the environment.

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